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Evaluation of pre-PCR processing approaches for enumeration of *Salmonella enterica* in naturally contaminated animal feed

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Aim

To evaluate 3 pre-PCR processing strategies:

1) flotation-qPCR (modified from [1])
2) MPN-PCR (modified from [2])
3) qualitative culture enrichment PCR [2]

for the detection and/or quantification of *Salmonella* in naturally contaminated soy bean meal.

Introduction

Animal feed might serve as a reservoir of *Salmonella* contributing to the spread into the food chain. Levels of *Salmonella* in feed samples are low, bacteria are unevenly distributed and stressed and could therefore be hard to recover using standard culture-based methods. Due to this, there is a need for accurate, sensitive, rapid and user-friendly sample preparation methods prior to molecular analyses. Moreover, to facilitate quantitative risk assessment in the feed production chain, there is a need to enumerate *Salmonella* in naturally contaminated feed.

Methods

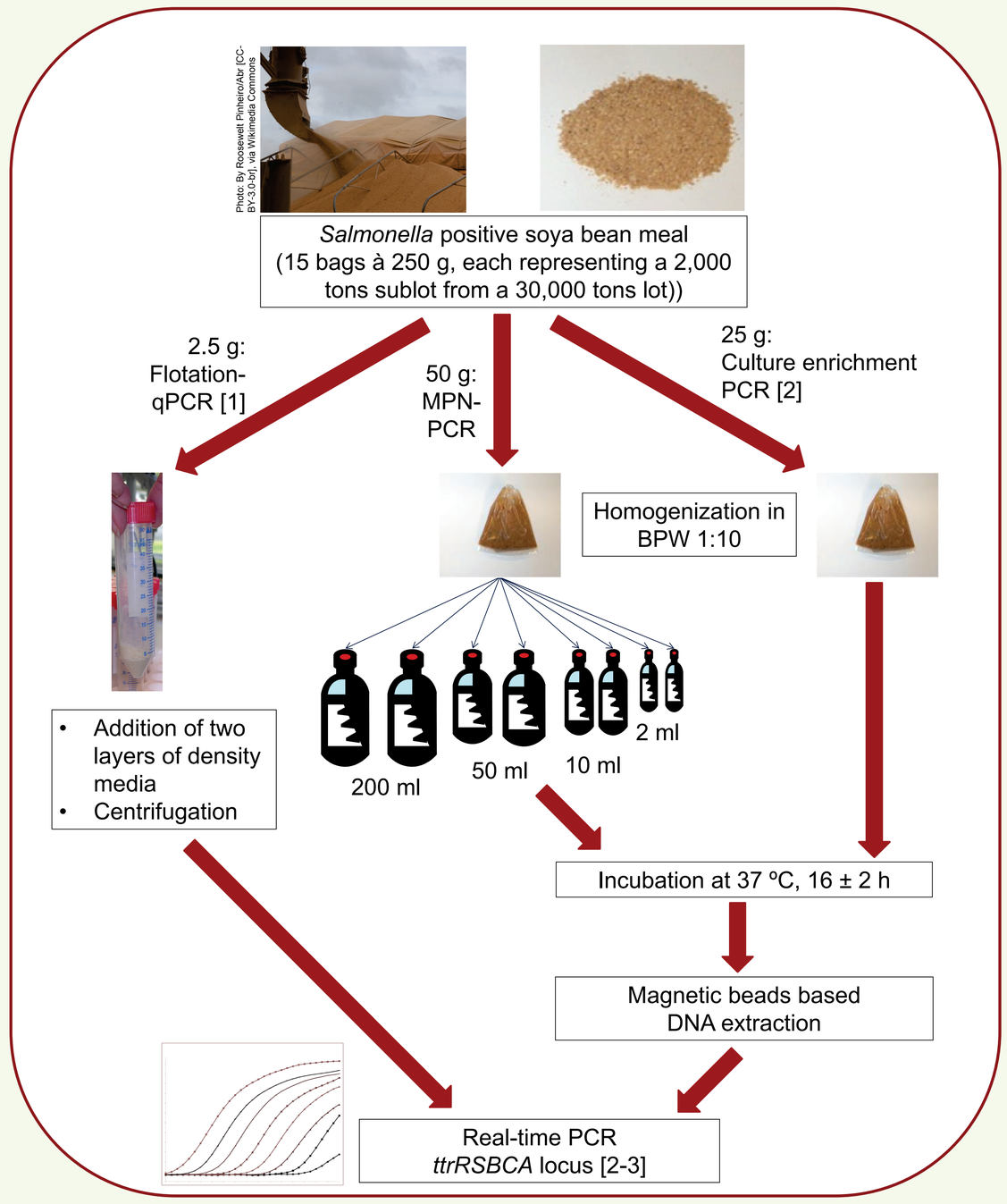

Figure 1. Overview of the three pre-PCR processing strategies that were investigated. Bags of naturally contaminated soya bean meal (n = 15) were analyzed in parallel with the three methods.

Conclusions

- The 3 methods provide possibilities to assess the prevalence of *Salmonella* in feed, as well as the numbers of culturable, and non-culturable cells
- Differences in results could be due to non-culturable *Salmonella* and/or a heterogeneous distribution of *Salmonella* in the feed

Results

Out of the 15 bags analyzed 6, 15 and 9 were positive for *Salmonella* with flotation-qPCR, MPN-PCR and culture enrichment PCR, respectively (Table 1).

Enumeration resulted in values of 1.8×10^2-7.8×10^3 CFU/g (flotation-qPCR) and 0.024 to >5.2 MPN/g (MPN-PCR) (Figure 2).

Table 1. Comparison of qualitative results with the three methods for the analysis of naturally contaminated soya bean meal samples.

Method	Qualitative result (+/-) for indicated bag with soya bean meal														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Flotation-qPCR	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-
MPN-PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Culture enrichment PCR	+	+	+	+	-	+	-	-	+	-	+	+	-	+	-

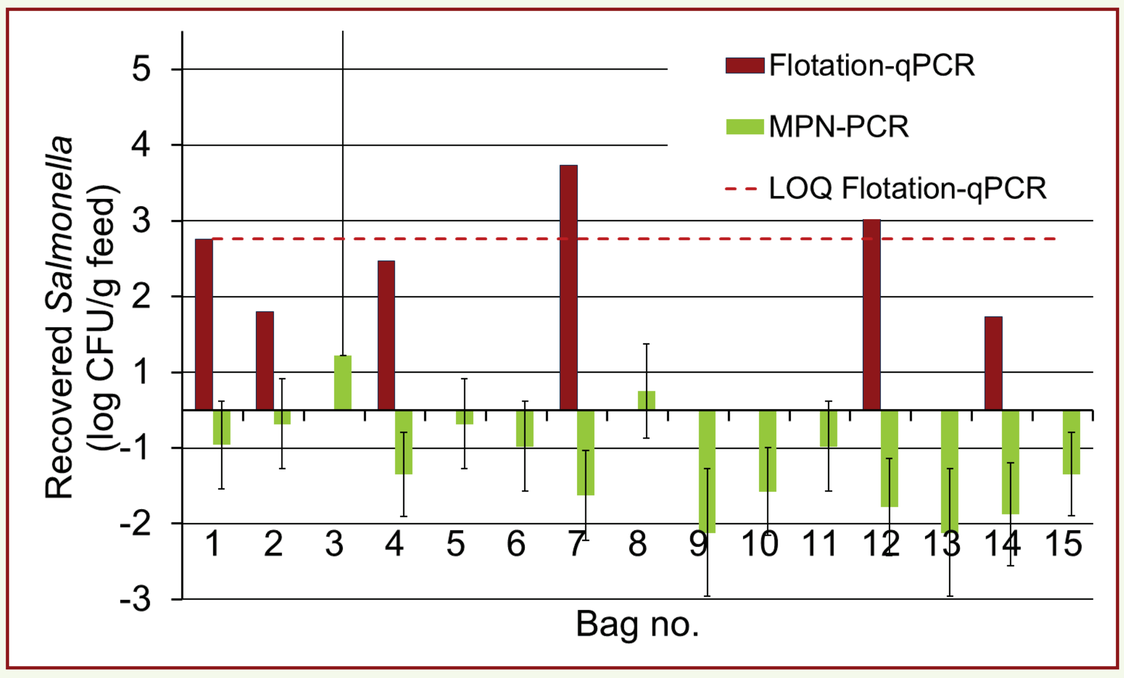

Figure 2. Comparison of enumeration results with flotation-qPCR and MPN-PCR for the analysis of naturally contaminated soya bean meal samples.

References

1. Löfström et al. (2011) IJFM 145 Suppl 1:S103-109
2. Löfström et al (2012) Vet. Microbiol. 158(3-4):431-435.
3. Malorny et al (2004) AEM, 70(12):7046-52.

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